Synopsis

Genomic and epidemiological profile of cervical cancer patients - identifying risk factors, pathways and novel variants through integrated survey and whole exome sequencing strategies

By

Sudha B

(Roll No. 19PHBTF001)

Supervisor

Dr. S. Sumathi

Professor

Department of Biochemistry, Biotechnology, and Bioinformatics

Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043

October 2024

Introduction

Cervical cancer is the fourth most frequent cancer in women, accounting for an expected 660,000 new cases and 350,000 deaths globally in 2022. In India, cervical cancer is the second largest cause of cancer in women (Singh et al., 2023). However, HPV vaccination for adolescents could prevent cervical cancer fatalities (Canfell et al., 2020). Preventing death from cervical cancer is achievable when vaccination, is given before beginning sexual activity; thus, it is thought to be more beneficial when given during early adolescence (Di Giuseppe et al., 2022).

The most common explanation for the more significant proportion of cervical cancer mortality in India was a lack of response to screening programs, warning signals, risk factors, and protective measures. Integrating awareness and information programs with cervical cancer prevention techniques, such as HPV screening and vaccination, would assist Indian women in reducing HPV infections and controlling cervical cancer (Taneja et al., 2021).

Epidemiological research indicates that up to 80% of sexually active women may be infected with the HPV virus (Chesson et al., 2014). While most viral infections are eliminated naturally by host immunity, a few remain and eventually cause cancer (Winer et al., 2019). Thus, cervical cancer is a rare complication of widespread HPV infections. Currently, no effective treatment exists for HPV persistence (de Sanjose et al., 2018). The prevention of HPV-related cervical cancer is dependent on expensive HPV vaccines and regular cervical screenings. Although vaccines have shown promising results in recent years, implementing universal HPV vaccination strategies is costly for developing countries, particularly in populous countries such as China and India, and multivalent vaccines cannot completely cover all major types of HPV infections in these countries (Yuan et al., 2021). Because cervical screening and vaccination programs impose a significant cost burden, many women in both developed and developing nations remain unprotected from HPV infections and linked cervical malignancies (Drolet et al., 2019).

The primary treatment options for cervical cancer are surgery and chemoradiotherapy. Recent advances in tailored therapies targeting specific somatic deviations have transformed cancer treatment Worldwide (Burmeister et al., 2022).

Identifying new therapy targets may improve current CC medications (Lou et al., 2015). As a result, it is critical to evaluate genetic alterations in cancer and classify tumors based on genetic variances. The recent development of targeted treatments has made it necessary and helpful to investigate genetic variants in various cancers and classify tumors based on molecular genotyping results. Clinical trials now include drugs that target mutant EGFR, RAF, HER2, ALK, RET, and FGFRs (Xiang et al., 2014).

Next-generation sequencing (NGS) is the primary method for determining the mutational landscape of numerous solid tumors and hematologic malignancies (Pant et al., 2014). Specific genetic variations in tumors can act as diagnostic, predictive, and prognostic markers. Driver mutations, on the other hand, offer tumors with selective growth benefits, and targeted therapies are being developed and tested in various clinical trials against some of these identified alterations. Cancer patients' treatment options are getting increasingly individualized and adaptive as personalized medicine advances. This indicates that the standard-of-care treatment for a tumor type is no longer the deciding factor in therapy selection but rather the somatic mutations identified (Garraway et al., 2013). The National Cancer Institute Molecular Analysis for Therapy Choice (NCI-MATCH) is an example of a precision medicine clinical trial in which patients are assigned to different treatment arms based on genetic alterations discovered in their tumors, regardless of histological tumor type (Tsimberidou et al., 2023).

NGS has recently begun substituting Sanger sequencing due to its ability to sequence many genes, the whole exome (protein-coding regions), or the entire genome simultaneously. As a result, NGS is used in various applications, including customized gene panels, whole exome sequencing (WES), and whole genome sequencing (WGS). Human genetics has advanced significantly thanks to next-generation sequencing (NGS), notably whole-exome sequencing (WES). WES, a cheap, high-throughput method, allows for the simultaneous assessment of the coding portions of over 20,000 genes or virtually every known coding exon in the human genome (Tetreault et al., 2015).

More importantly, WES has aided in the discovery of multiple novel cancer susceptibility genes and made it possible to detect somatic driver mutations in

sporadic malignancies (Rotunno et al., 2020). We can use whole genome sequencing (WGS) or WES to systematically catalog all somatic mutations found in a single tumor by comparing germline and tumor DNA from different patients.

Thus, by selecting genes with recurrent mutations across a group of tumors, novel driver genes and pathways can be found without making any presumptions about the underlying molecular mechanisms. The use of WES in this context revealed new and unexpected oncogenic pathways in cervical cancer, such as apoptosis, chromatin remodeling, ERBB2/PI3K/AKT/mTOR, and cell cycle pathways (Zammataro et al., 2019). As a result, we attempted to do whole exome sequencing to investigate the genomic alteration profile of squamous cell cervical cancer and identify the somatic nucleotide variant associated with squamous cell cervical cancer.

Null hypothesis: The combination of knowledge, awareness, socio-demographic factors, biological risk factors, and genetic variants does not significantly predict cervical cancer incidence and progression.

Alternate hypothesis: There is a significant correlation between knowledge, awareness, and prevention behaviours, and a combination of socio-demographic factors, biological risk factors, and specific genetic variants increase the risk of cervical cancer incidence and progression.

With this background, the following objectives are set:

- To elicit the knowledge, awareness and prevention behaviors related to cervical cancer through online response
- To ascertain the socio-demographic profile and biological risk factors of cervical cancer patients in hospital-based cohort study
- To identify genetic variants by whole exome sequencing and evaluate their pathogenicity and associated pathways in squamous cell cervical cancer patients
- To confirm the identified novel variants using Sanger sequencing

In order to meet the set objectives, our work was framed in four phases.

Phase I

The study's background highlights the significance of addressing cervical cancer, which claims over 67,000 lives annually in India due to a lack of knowledge about the disease's progression, screening, and prevention. HPV vaccines are crucial in controlling cervical cancer, and organizations like the US Advisory Committee on Immunization Practices and the World Health Organization (WHO) recommend vaccination for girls and females between 9 and 26 years old. However, implementation barriers in India include vaccine costs, health prioritization, and societal barriers.

The purpose of this study is to assess the knowledge and awareness about Human Papillomavirus (HPV) infections, cervical cancer, its signs and symptoms, and risk factors among women in South India. The study aims to identify socio-demographic factors that influence women's understanding of HPV and cervical cancer risk factors using circulation questionnaire among women.

Findings of Phase I

A study conducted among women in South India revealed alarming gaps in knowledge about HPV, cervical cancer, and its risk factors. Maximum number of participants being students and having a college-level education, participants lacked awareness about cervical cancer symptoms, prevention strategies, and risk factors. Only 63.1% of rural and 71.3% of urban participants knew about cervical cancer, while 74.5% of rural and 70.38% of urban participants were aware of HPV.

The study highlighted that educational sources and media are crucial in disseminating informations about cervical cancer. However, participants' knowledge about cervical cancer prevention strategies, such as Pap tests and availability of HPV vaccines, was limited. Only 18.6% of rural and 27.23% of urban participants knew about Pap tests, while 26.75% of rural and 19.76% of urban participants knew of HPV vaccines.

The majority of participants were unaware of cervical cancer symptoms, with 61.75% of rural and 53% of urban participants lacking knowledge. Regarding risk factors, HPV infection, multiple sexual partners, and weakened immune systems

were recognized. Still, many participants were unaware of other risk factors, such as smoking, long-term oral contraceptive use, and early pregnancy.

The study's findings underscores the need for targeted interventions to educate women about cervical cancer, its symptoms, and risk factors. Awareness and knowledge can enhance early detection, prevention, and treatment outcomes. Furthermore, incorporating HPV vaccination into national immunization programs and addressing societal and cultural barriers can significantly reduce cervical cancer incidences and mortality rates in India.

Overall, the study emphasizes the importance of education and awareness in combating cervical cancer and highlights the need for comprehensive and personalized approaches to address the disease's complex factors. Healthcare providers can offer more effective and less invasive options by predicting how patients may respond to different treatments, improving outcomes and minimizing side effects.

Next phase we attempted to see their factors in a hospital based cohort study.

Phase II

Hospital-based studies on cervical cancer patients aim to improve understanding of the disease, patient outcomes, and healthcare policy. These studies address epidemiological understanding, treatment effectiveness, healthcare planning, quality of life, and supportive care. They also drive research and innovation, addressing gaps in knowledge such as limited data on epidemiology, HPV vaccination effectiveness, and personalized treatment strategies.

This study received ethical approval from Sri Ramakrishna Hospital, Coimbatore, Tamil Nadu. Responses were collected voluntarily, and all information was handled with utmost confidentiality and anonymity. This hospital-based, descriptive cross-sectional study investigated cervical cancer patients treated at Sri Ramakrishna Hospital, Coimbatore, Tamil Nadu, India, between January 2020 and December 2022. The study included patients aged 29-85 years. Participants, comprising cervical cancer survivors and newly diagnosed cases, provided informed consent after being briefed on the study's objectives.

This study aims to investigate cervical cancer patients' socio-demographic profiles, experienced symptoms, and treatment methods, analyze statistically significant clinical variables, and address gaps in knowledge on epidemiology, HPV vaccination effectiveness, and personalized treatment strategies. Inform evidence-based practice, improve patient outcomes, enhance healthcare policy, and advance research.

Findings of Phase II

Cervical cancer is a significant health concern in Tamil Nadu, India, with a high prevalence among middle-aged and older women. A hospital-based study conducted in Sri Ramakrishna Hospital, Coimbatore, between January 2020 – December 2022 revealed that the median age of cervical cancer patients was 54.8 years, with the majority residing in rural areas. The study highlighted the importance of socio-demographic factors, clinical characteristics, and treatment patterns in informing effective management and prevention strategies.

The research showed that working women, particularly in rural areas, were at higher risk of cervical cancer due to occupational exposure to carcinogens, limited access to healthcare services, and lower socioeconomic status. Tobacco consumption, although a known risk factor, did not significantly impact cervical cancer risk in this study population. Comorbidities, such as diabetes and hypertension, were prevalent among patients.

Clinical characteristics revealed that abdominal pain, leucorrhoea, weight loss, and post-menopausal bleeding were common symptoms. Squamous cell carcinoma (SCC) accounted for 77.96% of cases, followed by adenocarcinoma (15.46%). Histological diagnosis and reporting were crucial for tailored treatment strategies.

Treatment patterns indicated that cisplatin-based combinations dominated combined therapy approaches. Monotherapy involves chemotherapy, primarily cisplatin and radiotherapy. Understanding these treatment patterns informs clinical decision-making and future research directions.

The study's findings emphasize the need for targeted interventions for rural working women, improved access to healthcare services, and enhanced histological

diagnosis and reporting. Exome sequencing of SCC samples can provide insights into genetic alterations, driving diagnostic and therapeutic strategies.

Phase III

Cervical cancer is a leading cancer among Indian women, with limited reports on its mutational landscape. WES is an effective tool for identifying novel variations, particularly in genetically heterogeneous diseases like cancer. This study utilizes Whole Exome Sequencing (WES) to unravel the genetic complexities of cervical cancer, specifically squamous cell carcinoma.

This study focused on five squamous cell cervical cancer patients (CC1-CC5) selected from the Oncology division of Sri Ramakrishna Hospital, Coimbatore. Participants were included based on strict criteria: histological confirmation of squamous cell cervical cancer, availability of biopsy samples, and provision of informed consent. Comprehensive patient profiles were compiled, encompassing demographic, clinical, and lifestyle factors, including age, histology type, nutritional status, presence of anemia and jaundice, blood type, symptoms, medical history, dietary habits, and comorbidities.

This study aims to contribute to understanding cervical cancer genetics, improve diagnostics, and inform personalized treatment strategies. WES has the potential to become a clinically feasible approach for patient diagnostics, enabling affordable and everyday clinical use.

Findings of Phase III

Five patients (CC1-CC5) diagnosed with squamous cell cervical cancer participated in this study. Their ages ranged from 52-70 years, with fair nutritional status and no anemia or jaundice. DNA was isolated from biopsy samples using the QIAamp DNA Mini Kit, yielding high-quality DNA with minimal protein contamination. The A260/280 ratio, an indicator of DNA purity, ranged from 1.80 to 1.85.

DNA libraries were prepared for next-generation sequencing (NGS). Quality control metrics revealed varying concentrations (23.4-56.6 ng/µl) and consistent insert sizes (433-458 bp) across samples. These metrics inform sequencing strategy

and optimization. Approximately 63.7 million high-quality reads were generated through whole exome sequencing (WES), with 97.7% passing quality control.

The resulting exome sequencing data were aligned to each sample's human reference genome (hg38). Somatic single-nucleotide variations (SNVs) were investigated in the five squamous cell cervical cancer samples. An allele frequency threshold of ≤0.05 yielded 6,658 SNVs, categorized into exons, UTRs, introns, and splice sites. Nonsynonymous mutations (2,389) were identified, altering protein function.

Of the variants identified in our study, 285 were found to match entries in ClinVar's database, indicating reported clinical relevance. Nonsynonymous single nucleotide polymorphisms (nsSNPs) were selected to identify important on protein structure and functions. Mutation spectrum analysis revealed distinct transition and transversion patterns, with G>A transitions (28.25% of total mutations) and G>C transversions being the most common (5.9% of total mutations).

These findings provide insights into the mutational landscape of cervical cancer especially squamous cell cervical cancer. The identified nonsynonymous variants may contribute to tumorigenesis and cancer progression, serving as potential biomarkers for diagnosis or prognosis. This study provides a comprehensive analysis of somatic mutations in squamous cell cervical cancer, shedding light on the genetic landscape of this disease.

The study results highlighted the importance of integrating genomic analysis into squamous cell cervical cancer research, enabling a better understanding of the disease's underlying mechanisms and potential vulnerabilities. By exploring the functional consequences of these SNVs, researchers can uncover new avenues for cancer diagnosis, treatment, and prevention. Although limited by the sample size, this study contributes to understanding squamous cell cervical cancer's genetic underpinnings, informing the development of personalized therapeutic strategies.

This study analyzed nonsynonymous variants in squamous cell cervical cancer to understand their potential impact on protein function. Renowned algorithms, including SIFT, PolyPhen-2, and Mutation Taster, were used to predict the effects of these variants. The majority of variants had deleterious SIFT scores

(<0.05), indicating harmful effects on protein function. PolyPhen-2 scores were predominantly high (>0.9), suggesting probably damaging effects.

The analysis revealed variants in multiple genes across different samples, suggesting shared genetic pathways may be disrupted in squamous cell cervical cancer. Notably, variants in the TTN gene were detected in multiple samples, implying a possible role in the disease. Variants in CEL and GUCA1B genes were also identified.

The selected variants encompassed variants in genes involved in diverse cellular processes, including cell adhesion and signaling, cytoskeleton organization, membrane transport, metabolism, and protein modification. High-confidence variants, including those in TTN, GUCA1B, ECEL1, and RHBDF2, were identified.

A comprehensive gene enrichment analysis provided insights into disrupted biological processes, cellular components, and molecular functions in squamous cell cervical cancer. Significant enrichment was observed in mitochondrion organization, protein localization, striated muscle contraction, lipid metabolic processes, and cardiac muscle cell development. The analysis highlighted cellular component associations, including cytosol, extracellular exosome, condensed nuclear chromosome, and brush border. Notably, 13 genes were linked to the cytosol, underscoring the critical role of cytosolic proteins in cervical cancer. Significant enrichment was observed at the molecular function level in ATP binding, actin filament binding, structural constituent of muscle, protein binding, and enzyme binding. Twenty-two genes were implicated in protein binding.

A protein-protein interaction network was constructed to decipher functional relationships among mutated genes. The network revealed a complex interplay between 22 seed nodes, 390 proteins, and 436 interactions. Essential genes emerged as hub genes, including TTN, RPN1, PLEC, ABCC2, and LBR.

Network topology analysis identified these genes as critical conduits facilitating information flow and functional coordination within the network. TTN exhibited an exceptionally high degree of centrality and betweenness centrality values.

The integration of mutated genes and their interacting partners with KEGG pathways unveiled significant enrichment in various biological processes, including Focal Adhesion, Protein Processing in Endoplasmic Reticulum, Viral Carcinogenesis, Leukocyte Trans-endothelial Migration, and Cellular Senescence. The Focal Adhesion pathway emerged as the most significantly enriched pathway, suggesting cell adhesion and cytoskeleton organization disruptions contribute to cancer progression. The Protein Processing in the Endoplasmic Reticulum pathway was also enriched considerably, implying impacts on protein homeostasis and cellular function.

To the best of our knowledge, this study identified novel mutations in six genes - POM121C, PRICKLE1, SRPX2, BRAT1, ALOX12B, and GLIS3 - substantially advancing our understanding of the genetic landscape of squamous cell cervical cancer. This study provides valuable insights into the molecular mechanisms underlying cervical cancer development and progression, highlighting potential therapeutic targets. The findings underscore the importance of integrating mutation spectrum analysis with functional predictions and pathway enrichment analysis.

Phase IV

Cervical cancer, particularly squamous cell carcinoma, is a complex and devastating disease with significant genetic components. Despite advances in cervical cancer research, the genetic basis of this disease remains poorly understood. Recent studies have highlighted the importance of genetic variations, including missense variants, in contributing to cervical cancer development and progression. Exome sequencing, a powerful tool for analyzing protein-coding regions of the genome, offers an opportunity to identify novel missense variants contributing to cervical cancer. Combining exome sequencing with traditional sequencing methods, such as Sanger sequencing, ensures data accuracy and reliability.

This study utilized exome and Sanger sequencing to identify and validate novel missense variants associated with squamous cell cervical cancer. The process involved exome sequencing to capture genetic variations, mutational analysis to prioritize variants, and Sanger sequencing validation to confirm novel variants. Existing research has identified several genetic variants associated with cervical cancer, but the genetic landscape of this disease remains incomplete. There is a

need for comprehensive genetic analysis to identify novel variants and understand their role in disease pathogenesis.

Findings of Phase IV

This study analyzed squamous cell cervical cancer samples using whole exome sequencing, revealing a set of genetic variants, including seven nonsynonymous mutations across six genes: POM121C, PRICKLE1, SRPX2, BRAT1, ALOX12B, and GLIS3. These variants were identified in samples CC1-CC5, with unique chromosome positions, reference, alternative bases, and predicted amino acid changes.

POM121C exhibited a threonine-to-proline change at position 317, while PRICKLE1 displayed an arginine-to-glutamine change at position 373. Similarly, SRPX2 showed an alanine-to-valine change at position 124, BRAT1 had a valine-to-isoleucine change at position 171, and ALOX12B presented a tyrosine-to-cysteine change at position 687. Interestingly, GLIS3 harbored an arginine-to-cysteine change at position 747 in two samples, CC4 and CC5.

These genes play critical roles in various biological processes, including nuclear pore complex formation, planar cell polarity signaling, RNA binding and splicing, cell growth regulation, arachidonic acid metabolism, and transcriptional regulation. The absence of dbSNP IDs suggests these variants may be novel or rare.

Predictive analysis using SIFT, PolyPhen-2, and Mutation Taster tools assessed the potential impact of these variants on protein function. The results indicated that the ALOX12B variant is likely deleterious and damaging, while GLIS3 variants may contribute to disease pathogenesis.

To validate these variants, specific primers were designed using Primer3Plus for PCR amplification and Sanger sequencing. Despite DNA quantity limitations, successful amplification was achieved for three of six genetic variants.

Sanger sequencing confirmed the presence of nonsynonymous mutations in POM121C, PRICKLE1, and GLIS3 genes. The p.Thr317Pro mutation in POM121C may disrupt nuclear pore complex assembly and function, while the p.Arg373Gln mutation in PRICKLE1 may impair planar cell polarity signaling. The p.Arg747Cys mutation in GLIS3 may disrupt transcriptional regulation and insulin signaling,

potentially contributing to diabetes and pancreatic disorders. Further investigation is necessary to understand the functional implications of these mutations and their role in disease pathogenesis.

The study's findings provide valuable insights into the genetic landscape of squamous cell cervical cancer, highlighting potential molecular mechanisms underlying disease development and progression. Identifying these genetic variants may have significant implications for diagnosis, treatment, and prevention.

Future studies should focus on the functional characterization of these variants, exploring their potential as biomarkers or therapeutic targets. Elucidating the molecular mechanisms underlying squamous cell cervical cancer will ultimately inform personalized medicine approaches, improving patient outcomes and quality of life.

Conclusion

A study in Tamil Nadu found that educated women lacked knowledge about Human Papillomavirus (HPV) infection, cervical cancer, and its risk factors. Only 18.6-27.23% knew about Pap tests, and 19.76-26.75% knew about HPV vaccines. Cervical cancer primarily affects middle-aged and older women in rural Tamil Nadu, with limited healthcare access and lower socioeconomic status increasing risk. Symptoms include abdominal pain, weight loss, and bleeding. Cisplatin-based combinations and chemotherapy/radiotherapy were the primary treatments. Our groundbreaking research has identified novel mutations in six key genes - POM121C, PRICKLE1, SRPX2, BRAT1, ALOX12B, and GLIS3 - that significantly contribute to the genetic etiology of squamous cell cervical cancer. This pioneering finding substantially advances our understanding of the complex genetic landscape underlying this devastating disease. These findings emphasize the need for better education and personalized medicine approaches to combat cervical cancer in South India. Future research should explore these genetic variants as potential biomarkers or therapeutic targets.

Reference

1. Singh, D., Vignat, J., Lorenzoni, V., Eslahi, M., Ginsburg, O., Lauby-Secretan, B., Arbyn, M., Basu, P., Bray, F., & Vaccarella, S. (2023). Global estimates of

incidence and mortality of cervical cancer in 2020: A baseline analysis of the WHO Global Cervical Cancer Elimination Initiative. *The Lancet Global Health*, *11*(2), e197-e206.

2. Canfell, K., Kim, J. J., Brisson, M., Keane, A., Simms, K. T., Caruana, M., Burger, E. A., Martin, D., Nguyen, D. T., Bénard, É., & Sy, S. (2020). Mortality impact of achieving WHO cervical cancer elimination targets: A comparative modelling analysis in 78 low-income and lower-middle-income countries. *The Lancet*, *395*(10224), 591-603.
3. Di Giuseppe, G., Folcarelli, L., Lanzano, R., Napolitano, F., & Pavia, M. (2022). HPV vaccination and cervical cancer screening: Assessing awareness, attitudes, and adherence in detained women. *Vaccines*, *10*(8), 1280.
4. Taneja, N., Chawla, B., Awasthi, A. A., Shrivastav, K. D., Jaggi, V. K., & Janardhanan, R. (2021). Knowledge, attitude, and practice on cervical cancer and screening among women in India: A review. *Cancer Control*, *28*, 10732748211010799.
5. Chesson, H. W., Dunne, E. F., Hariri, S., & Markowitz, L. E. (2014). The estimated lifetime probability of acquiring human papillomavirus in the United States. *Sexually Transmitted Diseases*, *41*(11), 660-664.
6. Winer, R. L., Lin, J., Tiro, J. A., Miglioretti, D. L., Beatty, T., Gao, H., Kimbel, K., Thayer, C., & Buist, D. S. (2019). Effect of mailed human papillomavirus test kits vs usual care reminders on cervical cancer screening uptake, precancer detection, and treatment: A randomized clinical trial. *JAMA Network Open*, *2*(11), e1914729.
7. de Sanjose, S., Brotons, M., & Pavon, M. A. (2018). The natural history of human papillomavirus infection. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *47*, 2-13.
8. Yuan, Y., Cai, X., Shen, F., & Ma, F. (2021). HPV post-infection microenvironment and cervical cancer. *Cancer Letters*, *497*, 243-254.
9. Drolet, M., Bénard, É., Pérez, N., Brisson, M., Ali, H., Boily, M. C., Baldo, V., Brassard, P., Brotherton, J. M., Callander, D., & Checchi, M. (2019). Population-level impact and herd effects following the introduction of human papillomavirus vaccination programmes: Updated systematic review and meta-analysis. *The Lancet*, *394*(10197), 497-509.

10. Burmeister, C. A., Khan, S. F., Schäfer, G., Mbatani, N., Adams, T., Moodley, J., & Prince, S. (2022). Cervical cancer therapies: Current challenges and future perspectives. *Tumour Virus Research*, *13*, 200238.
11. Lou, J., Zhang, X., Liu, J., Dong, L., Wu, Q., Yan, L., Xu, C., Wu, Q., & Wang, M. (2024). The prognostic value of radiological and pathological lymph node status in patients with cervical cancer who underwent neoadjuvant chemotherapy and followed hysterectomy. *Scientific Reports*, *14*(1), 2045.
12. Xiang, L., Jiang, W., Li, J., Shen, X., Yang, W., Yang, G., Wu, X., & Yang, H. (2015). PIK3CA mutation analysis in Chinese patients with surgically resected cervical cancer. *Scientific Reports*, *5*(1), 14035.
13. Pant, S., Weiner, R., & Marton, M. J. (2014). Navigating the rapids: The development of regulated next-generation sequencing-based clinical trial assays and companion diagnostics. *Frontiers in Oncology*, *4*, 78.
14. Garraway, L. A. (2013). Genomics-driven oncology: Framework for an emerging paradigm. *Journal of Clinical Oncology*, *31*(15), 1806-1814.
15. Tsimberidou, A. M., Kahle, M., Vo, H. H., Baysal, M. A., Johnson, A., & Meric-Bernstam, F. (2023). Molecular tumour boards—current and future considerations for precision oncology. *Nature Reviews Clinical Oncology*, *20*(12), 843-863.
16. Tetreault, M., Bareke, E., Nadaf, J., Alirezaie, N., & Majewski, J. (2015). Whole-exome sequencing as a diagnostic tool: Current challenges and future opportunities. *Expert Review of Molecular Diagnostics*, *15*(6), 749-760.
17. Rotunno, M., Barajas, R., Clyne, M., Hoover, E., Simonds, N. I., Lam, T. K., Mechanic, L. E., Goldstein, A. M., & Gillanders, E. M. (2020). A systematic literature review of whole exome and genome sequencing population studies of genetic susceptibility to cancer. *Cancer Epidemiology, Biomarkers & Prevention*, *29*(8), 1519-1534.
18. Zammataro, L., Lopez, S., Bellone, S., Pettinella, F., Bonazzoli, E., Perrone, E., Zhao, S., Menderes, G., Altwerger, G., Han, C., & Zeybek, B. (2019). Whole-exome sequencing of cervical carcinomas identifies activating ERBB2 and PIK3CA mutations as targets for combination therapy. *Proceedings of the National Academy of Sciences*, *116*(45), 22730-22736.